

WHAT IS CLAIMED IS:

- 1 1. A method for enzymatically processing nucleic acid molecules in a mixture that includes
2 a chaotropic agent, degraded and denatured proteins and RNA and DNA molecules freed
3 of bound proteins, comprising diluting said mixture with at least one aqueous reagent to
4 reduce the concentration of chaotropic agent to less than 0.05 M and subjecting at least
5 some of said RNA and DNA molecules to at least one enzymatic process without removal
6 of or isolation of said RNA and DNA molecules from each other or from the other
7 components of the reaction mixture.
- 1 2. The method according to claim 1, wherein the mixture is diluted to reduce the
2 concentration of chaotropic agent to less than 0.01M.
- 1 3. The method according to claim 1 or claim 2 wherein said mixture includes at least one
2 component selected from the group consisting of a cell-lysing detergent, a reducing
3 agent, a water-miscible solvent, a chelating agent, and a neutralizing buffer.
- 1 4. The method according to any of claim 1 to claim 3, wherein dilution is accomplished by
2 serial addition of at least two aqueous reagents.
- 1 5. The method according to any of claim 1 to claim 4 wherein said at least one enzymatic
2 process includes exponential nucleic acid amplification.
- 1 6. The method according to claim 5 wherein said amplification is a polymerase chain
2 reaction process.
- 1 7. The method according to claim 5 wherein said at least one enzymatic process includes
2 reverse transcription.
- 1 8. The method according to any of claim 1 to claim 7 wherein an enzyme for performing
2 said at least one enzymatic process is included in said at least one aqueous reagent.
- 1 9. The method according to claim 8 wherein said enzyme is selected from the group
2 consisting of a DNase, a reverse transcriptase, a DNA polymerase, and a glycosidase.

- 1 10. The method according to claim 8 wherein the dilution and the at least one enzymatic
2 process are performed in the same container.
- 1 11. The method according to claim 10 wherein the container is selected from the group
2 consisting of a tube, a microtiter plate and a microfluidic device.
- 1 12. The method according to any of claim 1 to claim 11 wherein said mixture is prepared by
2 incubating a sample containing protein-bound RNA and DNA molecules and a chaotropic
3 agent-containing disruption reagent at a concentration of chaotropic agent of at least
4 about 2 M.
- 1 13. The method according to claim 12 wherein incubation of the sample and disruption
2 reagent includes heating, whereby chaotropic agent is concentrated.
- 1 14. The method according to claim 13 wherein said heating is sufficient such that the
2 resulting mixture is at least semi-dry.
- 1 15. The method according to claim 14 wherein the resulting mixture is stored prior to use.
- 1 16. The method of any of claim 12 to claim 14 wherein incubating and dilution are carried
2 out in the same container.
- 1 17. The method according to any of claim 12 to claim 16 wherein said disruption reagent is a
2 dry reagent.
- 1 18. The method according to claim 19 wherein said dry disruption reagent is prepared from a
2 mixture that includes a water-miscible solvent.
- 1 19. The method according to claim 17 or claim 18 wherein said dry disruption reagent is
2 prepared from a mixture that includes at least one component selected from the group
3 consisting of a cell-lysing detergent, a reducing agent, a chelating agent and a
4 neutralizing buffer.
- 1 20. The method according to any of claim 17 to claim 19 wherein said dry reagent is adhered
2 to a surface of a container..

- 1 21. The method according to claim 20 wherein said surface is the inner surface of a tube, a
2 tube cap, a wall of a microtiter plate or a microtiter plate cover.
- 1 22. The method according to any of claim 12 to claim 21 wherein said sample comprises
2 from a fraction of a cell up to 200 cells.
- 1 23. The method according to any of claim 12 to claim 22 wherein the amount of disruption
2 reagent is sufficient to provide a 2 M-8 M concentration of the chaotropic agent in a
3 volume of 20 to 50 nl.
- 1 24. A device for use in processing a biological sample containing protein-bound RNA and
2 DNA molecules to free said molecules from proteins comprising a dried disruption
3 reagent including a chaotropic agent adhered to a surface of a container or container part.
- 1 25. The device according to claim 24, wherein said container or container part is selected
2 from the group of a tube, a tube cap, a microtiter plate and a cover for at least one well of
3 a microtiter plate.
- 1 26. The device according to claim 24 or claim 25, wherein said container or container part
2 contains multiple chambers connected by flow channels.
- 1 27. The device according to any of claim 24 to claim 26, wherein said dried disruption
2 reagent contains an amount of chaotropic agent that will produce a concentration of 2 M
3 – 8 M chaotropic agent when dissolved in 20 – 50 nl of water.
- 1 28. The device according to any of claim 24 to claim 27, wherein said dried disruption
2 reagent contains a detergent.
- 1 29. The device according to any of claim 24 to claim 28, wherein said dried disruption
2 reagent includes at least one component from the group of a reducing agent, a chelating
3 agent, a water-miscible solvent and a buffer.
- 1 30. A kit comprising at least one reagent useful for enzymatic processing of nucleic acids and
2 a device according to any of claim 24 to claim 29.
- 1 31. The kit according to claim 30 including primers and enzyme for reverse transcription.

- 1 32. The kit according to claim 31, including a DNase enzyme.
- 1 33. The kit according to any of claim 30 to claim 32, including nucleic acid amplification
2 reagents comprising at least DNA polymerase and amplification buffer.
- 1 34. The kit according to claim 33, including at least one pair of polymerase chain reaction
2 primers and at least one sequencing primer.
- 3 35. The kit according to claim 33, including at least detection reagent.
- 1 36. The kit according to claim 35, including at least one dual labeled fluorescent
2 hybridization probe.